Circular No. 551

December 1939 • Washington, D. C.

UNITED STATES DEPARTMENT OF AGRICULTURE

The Basis for Treatment of Products Where Fruitflies Are Involved as a Condition for Entry into the United States

By A. C. Baker, *principal entomologist, in charge, Division of Fruitfly Investigations, Bureau of Entomology and Plant Quarantine*[1]

CONTENTS

The purpose of this circular is to set forth the methods of study and the character of the experiments which form the basis for treatments for products where fruitflies are involved. Two experimental objectives are in view: (1) To assure no survival of eggs or larvae in the products treated; (2) to lighten the treatment requirement as much as possible, so that, while safety is still assured, risk to the products is reduced to a minimum. Two types of treatment will be discussed in illustration. One involves the use of low temperatures, or the refrigeration method of sterilization. The other involves the use of high temperatures, or the vapor-heat method of sterilization.

PROCEDURE

While these two methods differ in the factors operating in the methods themselves, the procedure adopted in determining the mortality resulting from their application is identical in the two cases. Large quantities of infested fruit or other products are subjected to the treatment, and the fruit is subsequently held over moist sand under normal conditions until all larvae remaining alive therein have emerged and pupated. The resulting puparia are counted. Before treatment the fruit is divided at random, and a part thereof is held as a control lot. The number of normal puparia resulting from this control lot is accepted as an estimate of the number that would have resulted from a treated lot of equal size had no treatment been applied.

[1] The experimental work on which this circular is based was done by A. C. Mason, of the laboratory of the Bureau in Honolulu, T. H., with O. C. McBride in charge of the laboratory program.

173185—39

Under such procedure the control lot must be sufficiently large to smooth out variability in mortality among larvae in individual fruits.

Since the percentage of mortality obtained under such circumstances is largely dependent on the magnitude of the population used, conclusions are based on the results from a large number of cases. It is customary to use, at a given temperature, from about 100,000 to more than 200,000 larvae.

The mortalities resulting at all exposure points at any given temperature are transformed to probits[2] and plotted against time expressed in logarithms, and a regression line drawn. The use of probits and logarithms or a logarithmic scale, as here used, converts the relationship between mortality and length of exposure to a linear one so that expected mortalities in probits for each exposure may be read from the line. The line is thus used to determine how closely expected mortalities at each exposure agree with those obtained, and this serves as an indication of the suitability of the estimate of populations in the treated fruits as derived from the population in the untreated control. All experimental values are determined independently on separate populations.

It will be noted that lines of two slopes are shown in most of the diagrams. This is a condition found in most mortality records and possibly represents a reflection of two acting mechanisms in the mortality process. The main concern is with the upper line.

SECURITY REQUIRED FOR RECOMMENDED TREATMENT

The security demanded as a basis for recommendation is determined by reading on the regression line the exposure coordinated with a probit of 9. In percentages this probit represents a mortality of 99.99683 percent, or a survival of approximately 32 out of 1,000,000.[3] In view of the large numbers involved in the experiments and the high probit values obtained, this is considered a satisfactory procedure and one easily understood by those not accustomed to experimentation and such representation of data. Although survival expectancy is of this order, the security is considered adequate in view of the fact that to propagate not only must larvae survive and pupate but adults of both sexes must successfully emerge. Emergence, moreover, must be in one location, for the sexes must find each other and mate before successful oviposition can occur. In view of the further fact that, insofar as infestation can be detected, only sound fruit is admitted to shipment, the possibility of so many larvae occurring in shipped fruit is exceedingly remote.

It sometimes happens, in an individual exposure at a given temperature, that the number of larvae estimated to be contained in the fruit treated is not sufficiently large to demonstrate survival at that

[2] Fisher and Yates, in Statistical Tables For Biological, Agricultural, and Medical Research, footnote to table IX, p. 38, define the term "probit" as follows: "The probit corresponding to a given percentage is the normal deviate (increased by 5 to avoid negative values) exceeded by this percentage of the population." For an explanation of the use of probits for toxicological data see Bliss, C. I., THE CALCULATION OF THE DOSAGE-MORTALITY CURVE. Ann. Appl. Biol. 22: 134–167, illus., 1935; Fisher, R. A., THE DESIGN OF EXPERIMENTS, ed. 2, 1937; and Fisher and Yates, op. cit., pp. 5–7.

[3] Pearson, Karl, ed. TABLES FOR STATISTICIANS AND BIOMETRICIANS. Ed. 3, pt. 1, illus. Cambridge, England. 1930. See table 2, p. 6.

exposure. This is because the estimate cannot be made until after the larvae have emerged from the control fruit and have pupated. The treatment may be well advanced or even completed by that time. In general such a situation exists only at the longer exposures, where the populations used must be high to demonstrate survival.

For example, in the series for 32° F. (fig. 1) the line crosses 10 days at a level which would indicate that 2 larvae would survive out of 10,000. The estimate of the population in the fruit used was 8,214 larvae. One survivor was obtained at the 10-day exposure, a result agreeing closely with the expectation. On the other hand, the line crosses 11 days at a level which would indicate 0.5 of a survivor out of 10,000 larvae. Obviously the treated fruit would have to contain 20,000 or more larvae in order that any survival could be looked for. But in this case, when the infestation estimate was obtained, it showed

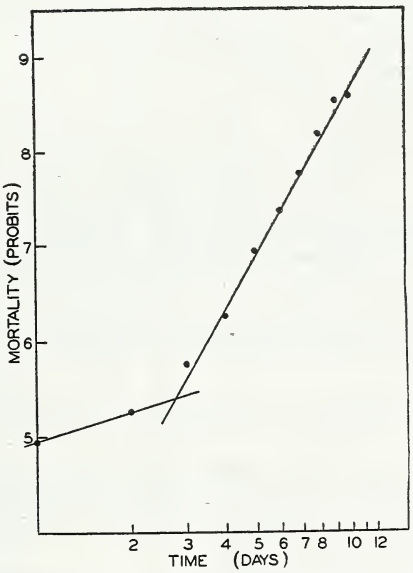

FIGURE 1.—Mortality of 136,131 larvae of *Ceratitis capitata* in fruit held at 32° F. Recommended treatment, 12 days at 32° F.

only 993 larvae in the treated fruit. The lack of survival at 11 days is therefore not valid experimental information.

This condition, arising when the numbers are too small to warrant estimated survival, accounts for the fact that the time between last survival and the exposure recommended for treatment may be different at different temperatures. The recommendation is made at the same security level, irrespective of such occurrences.

In contrast with conditions of this kind, attention may be called to the point for 6 hours for the vapor-heat treatment (fig. 6). The line here crossed at a level which would indicate an expected survival of 46.6, since the estimated population in the treated fruit at the 6-hour exposure was 33,290 larvae. The actual survival was 47. When the population which the fruit is estimated to contain proves to be sufficiently high to indicate an appreciable survival, as in this case, it becomes fairly certain that a valid survival figure will be obtained.

ILLUSTRATIVE RESULTS

In figures 1 to 5 and 7, diagrams are given for treatment results at temperatures of 32° to 36.5° F.; and in figure 6 a diagram is given of results at 110° under the vapor-heat treatment. In experiments with this last process the approach period is controlled so as to cover 8 hours. This period is the time required to raise the load from initial temperature to 110°. The numbers of larvae on which the diagrams are based are indicated in connection with the respective diagrams.

While many other data with various fruitflies have been accumulated, these diagrams will serve to illustrate the type of experimentation and the method of arriving at exposures for recommendation. They are thought to be instructive further for two reasons: (1) They show the similiarity of responses with different species of fruitflies. Thus, the line for 35° F. (fig. 4) represents data obtained with the melon fly (*Bactrocera cucurbitae* (Coq.)), whereas those for the other low temperatures represent data obtained with the Mediterranean fruitfly (*Ceratitis capitata* (Wied.)).

(2) The diagrams are of interest because of the information depicted in those for 34.5° and 35° F. The solid line shown in figure 3 is a line for 56,212 larvae, of which 37,760 were in treated kamani nuts (*Terminalia catappa* L.) The dotted line represents mortality when larvae from kamani are excluded. The difference between the mortality in these thin-pulped, rather dry nuts and that in typical fruits is at once apparent. A difference of the same nature has been demonstrated at other low temperatures, such as 32° and 35°, the situation at 35° being depicted in figure 7. Such differences show the importance of determining results with larvae living in products of somewhat the same general nature as those for which the recommendation is to be made.

OTHER CONSIDERATIONS

The following points are considered important in work of this kind: (1) Very large numbers are always used. (2) Determination is not made on the point of complete mortality. (3) Recommendation is made at a specific level of security.

It is possible that after exposures to low temperatures for a given period, approximating 10 days with *Ceratitis capitata*, the mortality rate changes. The determination of such a possibility will require many more data than are now available, and without proof of the existence of a change there is justification only in the assumption that the observed rate is continuous to the security points selected. Since a change, if it occurs, is in the direction of safety, the adoption of the continuous rate can in no way reduce security.

An explanation should here be made in connection with variation of temperature in experiments. For example, in the experiments at 36.5° F. temperature varied from 36° to 37°; but to be on the side of safety, recommendation is made at 36°. This added margin of safety involving certain fractions of a day is thought to be desirable at the higher temperatures where survival expectancies are greater. Future studies centered within individual degrees and upon them may make it feasible to propose recommendations on minimum hours rather than minimum days.

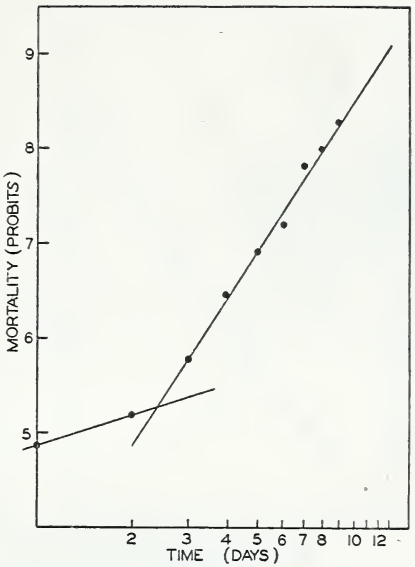

FIGURE 2.—Mortality of 78,190 larvae of *Ceratitis capitata* in fruit held at 33° F. Recommended treatment, 13 days at 33°.

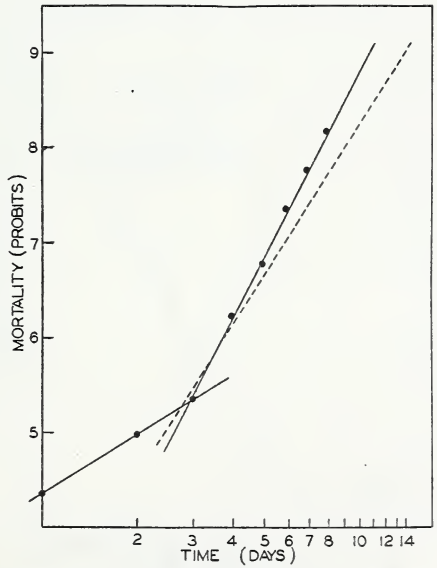

FIGURE 3.—Mortality of 56,212 larvae of *Ceratitis capitata* in fruit held at 34.5° F.: solid line, larvae in all fruits; dotted line, larvae in fruits other than kamani nuts (*Terminalia catappa*). Recommended treatment, 14 days at 34°.

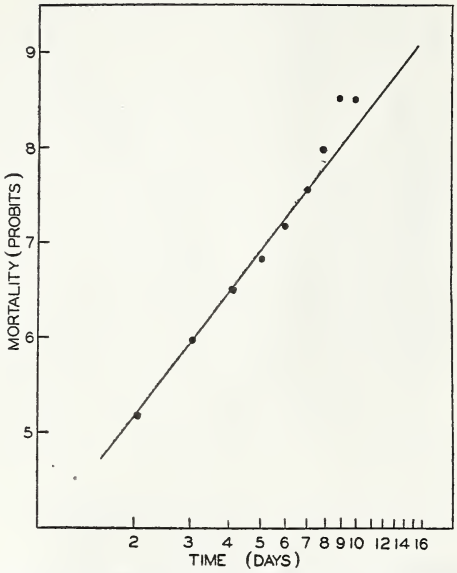

FIGURE 4.—Mortality of 72,213 larvae of *Bactrocera cucurbitae* in products held at 35° F. Recommended treatment, 15 days at 35°.

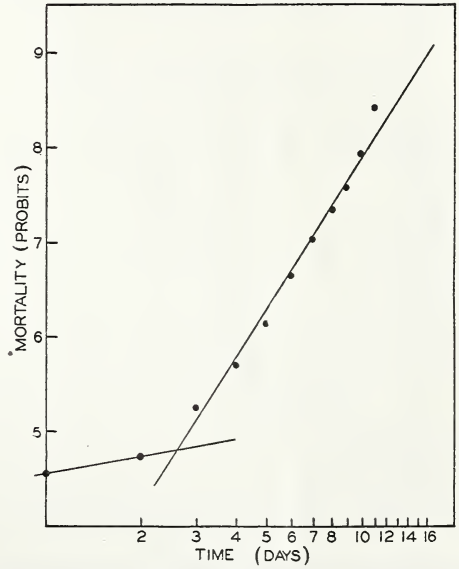

FIGURE 5.—Mortality of 90,826 larvae of *Ceratitis capitata* in fruit held at 36.5° F. Recommended treatment, 16 days at 36°.

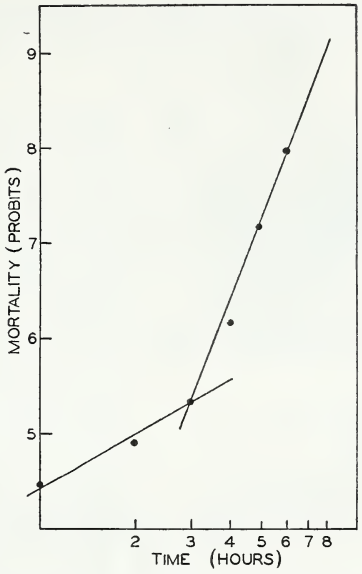

FIGURE 6.—Mortality of 110,873 larvae of *Ceratitis capitata* in fruit treated for 8 hours at 110° F. by the vapor-heat method. Recommended treatment, 8 hours at 110° by this method.

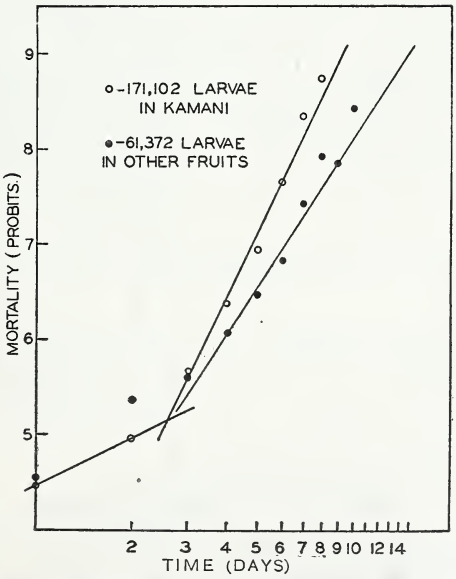

FIGURE 7.—Mortality of larvae of *Ceratitis capitata* in fruit held at 35° F.: open circles, larvae in kamani nuts (*Terminalia catappa*); closed circles, larvae in other fruits. Recommended treatment, 15 days at 35°.

ORGANIZATION OF THE UNITED STATES DEPARTMENT OF AGRICULTURE WHEN THIS PUBLICATION WAS LAST PRINTED

Secretary of Agriculture	HENRY A. WALLACE.
Under Secretary	M. L. WILSON.
Assistant Secretary	HARRY L. BROWN.
Director of Information	M. S. EISENHOWER.
Director of Extension Work	C. W. WARBURTON.
Director of Finance	W. A. JUMP.
Director of Personnel	ROY F. HENDRICKSON.
Director of Research	JAMES T. JARDINE.
Director of Marketing and Regulatory Work	A. G. BLACK.
Solicitor	MASTIN G. WHITE.
Land Use Coordinator	M. S. EISENHOWER.
Office of Plant and Operations	ARTHUR B. THATCHER, *Chief.*
Office of C. C. C. Activities	FRED W. MORRELL, *Chief.*
Office of Experiment Stations	JAMES T. JARDINE, *Chief.*
Office of Foreign Agricultural Relations	LESLIE A. WHEELER, *Director.*
Agricultural Adjustment Administration	R. M. EVANS, *Administrator.*
Bureau of Agricultural Chemistry and Engineering.	HENRY G. KNIGHT, *Chief.*
Bureau of Agricultural Economics	H. R. TOLLEY, *Chief.*
Agricultural Marketing Service	C. W. KITCHEN, *Chief.*
Bureau of Animal Industry	JOHN R. MOHLER, *Chief.*
Commodity Credit Corporation	CARL B. ROBBINS, *President.*
Commodity Exchange Administration	J. W. T. DUVEL, *Chief.*
Bureau of Dairy Industry	O. E. REED, *Chief.*
Bureau of Entomology and Plant Quarantine	LEE A. STRONG, *Chief.*
Farm Security Administration	W. W. ALEXANDER, *Administrator.*
Federal Crop Insurance Corporation	LEROY K. SMITH, *Manager.*
Federal Surplus Commodities Corporation	MILO R. PERKINS, *President.*
Food and Drug Administration	WALTER G. CAMPBELL, *Chief.*
Forest Service	FERDINAND A. SILCOX, *Chief.*
Bureau of Home Economics	LOUISE STANLEY, *Chief.*
Library	CLARIBEL R. BARNETT, *Librarian.*
Division of Marketing and Marketing Agreements.	MILO R. PERKINS, *In Charge.*
Bureau of Plant Industry	E. C. AUCHTER, *Chief.*
Rural Electrification Administration	HARRY SLATTERY, *Administrator.*
Soil Conservation Service	H. H. BENNETT, *Chief.*
Sugar Division	JOSHUA BERNHARDT, *Chief.*
Weather Bureau	FRANCIS W. REICHELDERFER, *Chief.*

This circular is a contribution from

Bureau of Entomology and Plant Quarantine	LEE A. STRONG, *Chief.*
Division of Fruitfly Investigations	A. C. BAKER, *Principal Entomologist, in Charge.*

U. S. GOVERNMENT PRINTING OFFICE: 1939

For sale by the Superintendent of Documents, Washington, D. C. - - - - Price 5 cents